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Research article

Carbon dioxide dynamics from sediment, sediment-water interface and overlying water in the aquaculture shrimp ponds in subtropical estuaries, southeast China

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ABSTRACT

Aquaculture ponds can emit a large amount carbon dioxide (CO_2), with the consequence of exacerbating global climate change. Many studies about CO_2 dynamics across the water-air interface, but CO_2 in sediment and overlying water received relative less attention. In this study, CO_2 concentration in sediment porewater, the diffusive CO_2 fluxes across the sediment-water interface (SWI), and the CO_2 production rates in the overlying water (CO_{2_WP}) were determined in the shrimp ponds in the Min River Estuary (MRE) and Jiulong River Estuary (JRE), southeast China, to analyze the dynamics of CO_2 among different growth stages of shrimps. Our results showed large variations in porewater CO_2 concentrations, CO_2 diffusive fluxes and CO_{2_WP} rates among different growth stages, with markedly larger values in the middle stage of shrimp growth. The temporal variation of CO_2 in both estuarine ponds followed closely the seasonal change of temperature. The internal CO_2 production (CO_{2_IP}) in these ponds was dominated by sediments. A significantly larger mean porewater CO_2 concentrations, diffusive fluxes and production rate were observed in the MRE ponds than those in the JRE ponds, which could be attributed to the lower water salinity and a larger source of carbon substrates in the former estuary. Considering a total surface area of $6.63 \times 10^3\ km^2$ across the mariculture ponds in subtropical estuaries, it is estimated conservatively that approximately 100 Gigagram (Gg) of dissolved organic carbon and 190 Gg of dissolved inorganic carbon were transported annually from the mariculture ponds into China's coastal areas. Because of the substantial supply of dissolved carbon, the adjacent coastal waters receiving effluent discharge from the mariculture ponds could become “hotspots” of CO_2 emissions. Our results highlight the role of aquaculture pond as a major CO_2 source in China's coastal areas, and effective actions are needed to alleviate the greenhouse gas (GHG) emissions from these ponds.

1. Introduction

The increasing worldwide concerns over global climate change and its effects on ecosystem and human society call for a better understanding of the magnitude of greenhouse gas (GHG) emissions (Tong et al., 2010; Yang et al., 2011; Yang and Flower, 2012). Carbon dioxide (CO_2) is one of the most potent GHGs, accounting for nearly 60% of the overall radiative forcing of the atmosphere (Mosier, 1998; Myhre et al., 2013). Global atmospheric CO_2 concentration has increased from 280 ppm in 1750 to 405 ppm in 2017, exceeding the pre-industrial levels by about 40% (World Meteorological Organization, 2018). Aquatic ecosystems (e.g. lakes, reservoirs, rivers, and others) are known to be

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Nomenclature

GHGs	Greenhouse gases
Gg	Gigagram
DOC	Dissolved organic carbon
DIC	Dissolved inorganic carbon
TC	Total carbon
Chl-*a*	Chlorophyll *a*
SWI	Sediment-water interface
MRE	Min River Estuary
JRE	Jiulong River Estuary
CO_{2_WP}	CO_2 production rates in the overlying water
CO_{2_IP}	Internal CO_2 production
CO_{2_SP}	CO_2 production at the sediment by microbial mineralization
CO_{2_PP}	CO_2 production in the water column by photochemical mineralization
CO_{2_SR}	CO_2 production in the water column by heterotrophic respiration of shrimps
ANOVA	Two-way analysis of variance
RMANOVA	Repeated measures analysis of variance
PCA	Principal component analysis

important sources of atmospheric CO_2. Earlier estimates indicate that inland freshwaters could emit CO_2 in the order of 1.4 Pg C $year^{-1}$ (Tranvik et al., 2009), equivalent to approximately 55% of the terrestrial carbon sink (Raymond et al., 2013; Tangen et al., 2016). Yet, accurate estimate of the regional and global CO_2 budgets remains challenging because of the large uncertainty of aquatic CO_2 emissions in relation to the limited field measurements. Shallow waters, including aquaculture ponds, have recently been highlighted as key hotspots for CO_2 emissions (Holgerson and Raymond, 2016; Yang et al., 2018b). Quantifying the potential CO_2 sources in various aquatic ecosystems has become one of the top priorities for improving the prediction of future CO_2 emission.

In response to the urgent need of climate change mitigation, there has been an increasing number of studies in recent years to explain the impacts of aquaculture systems on carbon cycle (e.g. Chen et al., 2015; Chen et al., 2016; Sidik and Lovelock, 2013; Yang et al., 2018b). However, the majority of these studies only focused on CO_2 fluxes across the water-air interface of aquaculture ponds, with little attention on the CO_2 fluxes across the sediment-water interface (SWI) (Xiong et al., 2017). CO_2 production can takes place either in anaerobic sediments or in aerobic water columns (Gruca-Rokosz et al., 2011; Xing et al., 2005); unfortunately, there is a lack of research on CO_2 production rates in the aerobic water column of aquaculture ponds. It is important to note that CO_2 fluxes across the water-air interface are not necessarily equivalent to the CO_2 fluxes across the SWI, due to the fact the actual proportion of CO_2 emitted to the atmosphere is also influenced by microbiological processes in water column (Gruca-Rokosz and Tomaszek, 2015; Xing et al., 2006; Yang et al., 2008). Therefore, studying the CO_2 dynamics across the SWI and quantifying the CO_2 production rates in the overlying water is crucial to improve our understanding of the overall carbon balance of aquaculture ponds and its resulting impacts on global warming.

According to the recent statistics, approximately 90% of the global aquaculture production occurs in Asia (FAO, 2014). In particular, China has the world's largest mariculture industry (Gu et al., 2017a, 2017b), with a total mariculture pond area and production of 2.57×10^4 km^2 and 2.30×10^9 kg, respectively in 2015, which comprise approximately 30% of the global total pond area and 60% of the global aquaculture production amount (Chen et al., 2016). Land-based aquaculture is one of dominant approaches of mariculture shrimp production (FAO, 2014). Mariculture ponds are generally semi-artificial ecosystems, with a large amount of organic matter supply from daily input of feeds (Chen et al., 2015; Yang et al., 2018a). The decomposition of organic matter from residual feeds and feces in these ponds can stimulate CO_2 production and emissions (Burford et al., 2003; Chen et al., 2016). Aquaculture ponds are inherently heterogeneous over spatiotemporal scales due to the changes in topographic features, environmental conditions, and tidal fluctuations, leading to large uncertainties in the calculation of CO_2 production and emissions. Unfortunately, the spatiotemporal variability of CO_2 production and emissions from

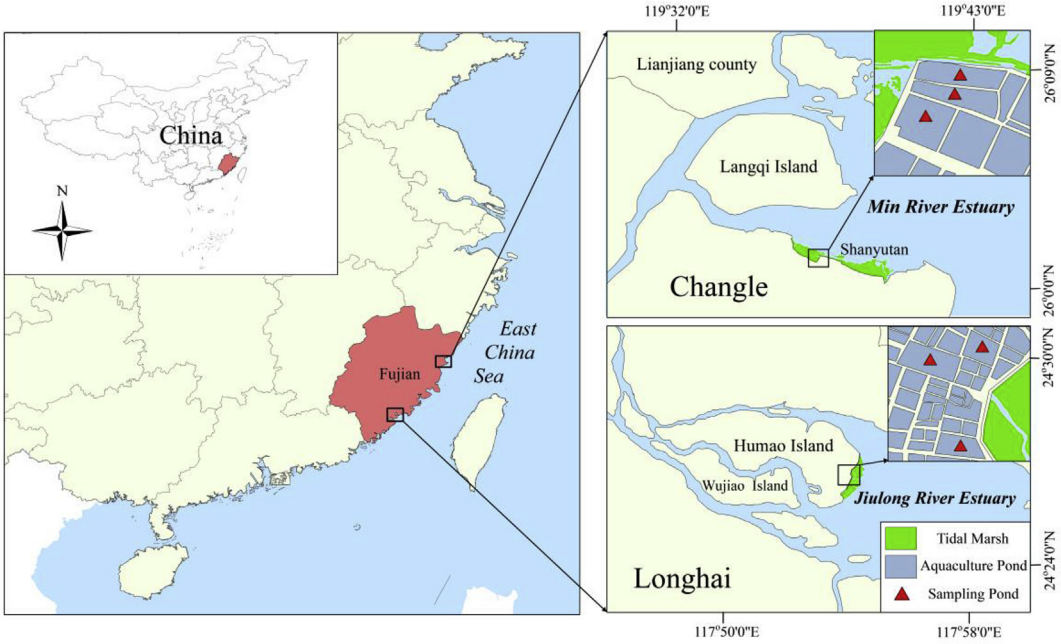

Fig. 1. Locations of the sampling sites in the subtropical aquaculture shrimp ponds in the Min River Estuary (MRE) and Jiulong River Estuary (JRE), Fujian, southeast China.

mariculture ponds are nearly unknown, which could cause biases in the estimation of the contribution of mariculture activities to radiative forcing and global. In the current research, we aim to fill these knowledge gaps by analyzing the CO_2 dynamics in aquaculture ponds between two subtropical estuaries in Fujian Province, which is one of the main distribution centers of shrimp produce in China.

2. Materials and methods

2.1. Study area description

This study was conducted in the Min River Estuary (MRE) and Jiulong River Estuary (JRE) located in southeast China (Fig. 1) (Yang et al., 2018a,b). The MRE has a typically subtropical monsoon climate, warm and wet in summer, with annual precipitation of 1350 mm and annual mean temperature of 19.6 °C (Tong et al., 2010). The JRE is in the subtropical oceanic climate zone. The mean annual rainfall is 1371 mm and annual air temperature is 21.0 °C (Wang et al., 2013). Both estuaries receive a greater amount of precipitation during the period between May and September owing to the southeast monsoon. Tides in both estuaries are semi-diurnal. The surface wetland soil is submerged for approximately 7 h during a 24 h cycle. The mean salinities of tidal water in MRE and JRE are approximately 4.2 ± 2.5 ppt (Tong et al., 2012) and 21.3 ± 2.9 ppt, respectively. Shrimp pond, one of the main landscapes in the estuarine zones, was mostly created by removing marsh vegetation.

2.2. Shrimp pond system and management

Because the optimal water temperatures to culture shrimp (*Litopenaeus vannamei*) are 22–35 °C, only one crop of shrimp could be produced annually at MRE and JRE (Yang et al., 2017a). The shrimp production cycle began in the second half of May and lasted for six months. Before the shrimp production, the ponds were filled with salt water pumped from an adjacent estuary. To prevent shrimp's predators and competitors, the water was passed through a 2-mm mesh bag (Guerrero-Galván et al., 1999; Yang et al., 2017a). Freshwater was added into the ponds in rainy days. After shrimp harvesting, water was discharged from the pond spillways. During the culture period, water levels in shrimp ponds ranged 1.1–1.5 m and 1.3–1.8 m in MRE and JRE, respectively.

Shrimps were fed with artificial feeds containing 42% crude protein (Yuehai™, China) in the morning (07:00) and afternoon (16:00) by direct application on the boat. According to water quality, pond management practices, and shrimp weight, the shrimp growth cycle was divided into three stages: initial, middle, and final stages (Yang et al., 2017a). Three to five 1500-W paddlewheel aerators were operated four times daily (07:00–09:00, 12:00–14:00, 18:00–20:00, and 00:00–03:00) in the ponds. Detailed information on the shrimp pond systems and management practices was reported in a previous study (Yang et al., 2017a). To analyze CO_2 dynamics in the culture period, water and sediment were sampled from three representative shrimp ponds at Shanyutan Wetland in MRE and the Humao Island in JRE, respectively (Fig. 1). Basic details of the selected mariculture ponds in the two estuaries are presented in Supporting Information in a previous study (Yang et al., 2018b).

2.3. Collection and analysis of water and sediment samples

2.3.1. Collection and analysis of water samples

Field sampling campaigns were performed in June, August, and October 2015 which represented three culture stages (initial, middle, and final). Sampling was performed on different but consecutive days (less than six days between the two estuaries) in any sampling campaign. Overlying water samples were collected from three sites in each pond. Overlying water was sampled at approximately 5 cm above the bottom sediments using a 5-L Niskin water sampler. Water samples were stored in an ice box, and transported to the laboratory within 4 h for incubation and measurement of water quality parameters within one week (Yang et al., 2017a). A portion of the water samples was filtered through a 0.45 μm cellulose acetate filter (Biotrans™ nylon membranes). The filtrates were subsequently analyzed for dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) using a Shimadzu total organic carbon analyzer (TOC-V). Chl-*a* concentrations in water samples were determined using a UV–visible spectrophotometer (Shimadzu UV-2450, Japan) following the methods of Jeffrey and Humphrey (1975) and Yang et al. (2017a). In addition, during each sampling campaign, the overlying water temperature and pH were determined using a pH/mV/Temp system (IQ150, IQ Scientific Instruments, USA), the salinity was measured using a salinity meter (Eutech Instruments-Salt6, USA) and DO was measured using a multi-parameter water quality meter (HORIBA, Japan).

2.3.2. Collection and analysis of sediment samples

Four intact sediment cores were sampled at each of the triplicate sites in each pond using a surface-operated coring device (Core-60, Austria). The device is composed of a core cylinder, a plexiglas tube (30 cm length, 6 cm internal diameter) and a one-way check valve which can preserve the integrity of 15 cm sediment and 15 cm overlying water (Yang et al., 2017a). The cores were sealed, stored vertically in an ice box, and transported to the laboratory within 4 h. In the laboratory, the four replicate sediment cores collected at each site were used separately for incubation experiments, and the measurement of sediment physicochemical properties, dissolved CO_2 concentrations, and physicochemical variables of sediment porewater. Sediment temperature were measured using a handheld pH/mV/temperature meter (IQ150, IQ Scientific Instruments, USA). Sediment porosity (Φ) was calculated based on the water content of sediment determined by weight loss after drying at 105 °C for 24 h (Zhang et al., 2013). Applying a soil-to-water ratio of 1:2.5 (w/v), sediment pH was determined using a pH meter (Orion 868, USA). After freeze drying, homogenization and grinding to fine power (Sun et al., 2013), sediment was analyzed for total carbon (TC) using an elemental analyzer (Elementar Vario MAX CN, Germany). Porewater was extracted from the bulk sediment by centrifugation at 5000 rpm for 10 min (Cence® L550, De Vittor et al., 2012). The extracted porewater samples were divided into two portions. One portion of the porewater samples was filtered through 0.45 μm pore size cellulose acetate filters (Biotrans™ nylon membranes) (De Vittor et al., 2012) and the filtrates were analyzed for DOC and DIC concentrations, while the other unfiltered portion was measured for salinity using a Eutech Instruments-Salt6 salinity meter.

To measure the dissolved CO_2 concentrations in sediment porewater, 6 cm^3 of sediment subsamples were collected in duplicate with 10 mL cut-off syringes and sealed in serum vials containing 24 mL of CO_2-free water and 0.5 mL of saturated $HgCl_2$ solution. The mixtures were shaken to achieve gas equilibrium between the slurry and the headspace (Dutta et al., 2015). Finally, the headspace CO_2 concentration was analyzed using a gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan). The CO_2 concentration in porewater (C, mg CO_2 L^{-1}) was calculated using the following equation (Ding et al., 2010; Johnson et al., 1990):

$$C = (C_h/22.4) \times [(\beta \times R \times T)/22.4 + (V_h/V_p)] \times (M/1000) \quad (1)$$

where C_h is the CO_2 concentration in vial headspace (mL L^{-1}); β is the Bunsen solubility coefficient for CO_2 (L L^{-1}) (Wiesenburg and Guinasso, 1979); R is the gas constant (0.0814); T is the room temperature (°C); M is molar mass of CO_2 (mg mol^{-1}); and V_h and V_p are the volumes of vial headspace volume (mL) and water sample (mL), respectively.

2.4. Laboratory incubation for the determination of CO_2 production and flux rates

The CO_2 production rates in the overlying water and CO_2 fluxes across the SWI were determined by *ex situ* incubation. The incubation device was constructed following the guide of Cowan and Boynton (1996), Chen et al. (2015), and Xiong et al. (2017). Basic details of the incubation device are also presented in Yang et al. (2017a). In brief, intact core samples containing equal volumes of sediments and overlying water were transported to the laboratory within 4 h after collection and placed in incubation chambers for 2 h to re-establish the equilibrium conditions (Xiong et al., 2017). After reaching an equilibrium, the incubation chambers were carefully filled with overlying water using a rubber pipe (Yang et al., 2017a), with special attention being paid to maintain a sufficiently low water flow rate to avoid any disturbance of the sediment surface. After filling the incubation chambers with overlying water, the cores were sealed with a Teflon plunger equipped with inlet and outlet tubes. The overlying water was continuously bubbled with air to simulate the *in situ* oxic conditions of water above the sediment (Mu et al., 2017), and overlying water were stirred during incubation. The chambers were then incubated in a temperature-regulated incubator device (QHZ-98A, China) for 9 h (Yang et al., 2017a). The incubation temperature was set to be the same as the field temperature (MRE: 22.5, 28.5, and 22.5 °C in June, August, and October, respectively; JRE: 25.5, 29.0, and 26.5 °C in June, August, and October, respectively). 60 mL of water samples were withdrawn from each chamber near the SWI using a 100 mL plastic syringe at 0 h, 3 h, 6 h, and 9 h of the incubation period. Subsequently, water samples were transferred to headspace vials to determine dissolved CO_2 concentrations using a gas chromatograph (Shimadzu GC-2010, Japan) based on the gas-stripping method (Zhang et al., 2010a). After each water sampling, the same volume of field-collected overlying water was introduced into the chamber to replace the sampled water and maintain the total volume of the water column in the incubation chamber. In addition, we incubated bottom water without any sediment under the same conditions in a separate chamber to estimate the CO_2 production rate in bottom water.

Dissolved CO_2 concentration in water samples was estimated by applying Henry's law and taking into account the dependence of gas solubility on water temperature and salinity (Wanninkhof, 1992). The CO_2 flux across the sediment-water interface (SWI) (mg m^{-2} h^{-1}), and CO_2 production rate in bottom water (CO_{2_WP}, mg m^{-2} h^{-1}) were calculated based on the CO_2 concentration changes in the water column over incubation time (Equation (2)) (Xiong et al., 2017; Zheng et al., 2009):

$$CO_2\text{flux}(\text{or } CO_{2_WP}) = \frac{dc}{dt} \times V \times S \times (M/1000) \quad (2)$$

where $\frac{dc}{dt}$ is the rate of change in CO_2 concentrations in the overlying water (mmol L^{-1} h^{-1}); V is the volume of overlying water in the incubation chamber (L); S is the cross-sectional area of the sediment core (m^2); and M is molar mass of CO_2 (mg mol^{-1}). Positive values of CO_2 fluxes indicate a net CO_2 release from sediments into the water column, whereas negative values indicate a net CO_2 uptake by sediments from the water column.

2.5. Statistical analysis

Two-way analysis of variance (ANOVA) was conducted to analyze the influences of estuaries and culture stages and the interaction between the two factors on sediment porewater CO_2 concentrations, CO_2 flux across the SWI, and CO_{2_WP}. Repeated measures analysis of variance (RMANOVA) was operated to examine the differences in environmental variables of shrimp ponds between these two estuaries during the study period, with the data collected in a given estuary over the three stages of shrimp growth being the repeated measures. Pearson correlation analysis was conducted to estimate the relationships (1) between porewater CO_2 concentrations, CO_{2_WP}, or CO_2 flux and environmental variables, and (2) between CO_2 fluxes and the gradient of CO_2 concentrations in both sediment porewater and the overlying water. Principal component analysis (PCA) was also performed to analyze relationships among the CO_2 production rates (or CO_2 fluxes) and observed environmental parameters and to show their pattern at different aquaculture stages. A stepwise regression analysis was further

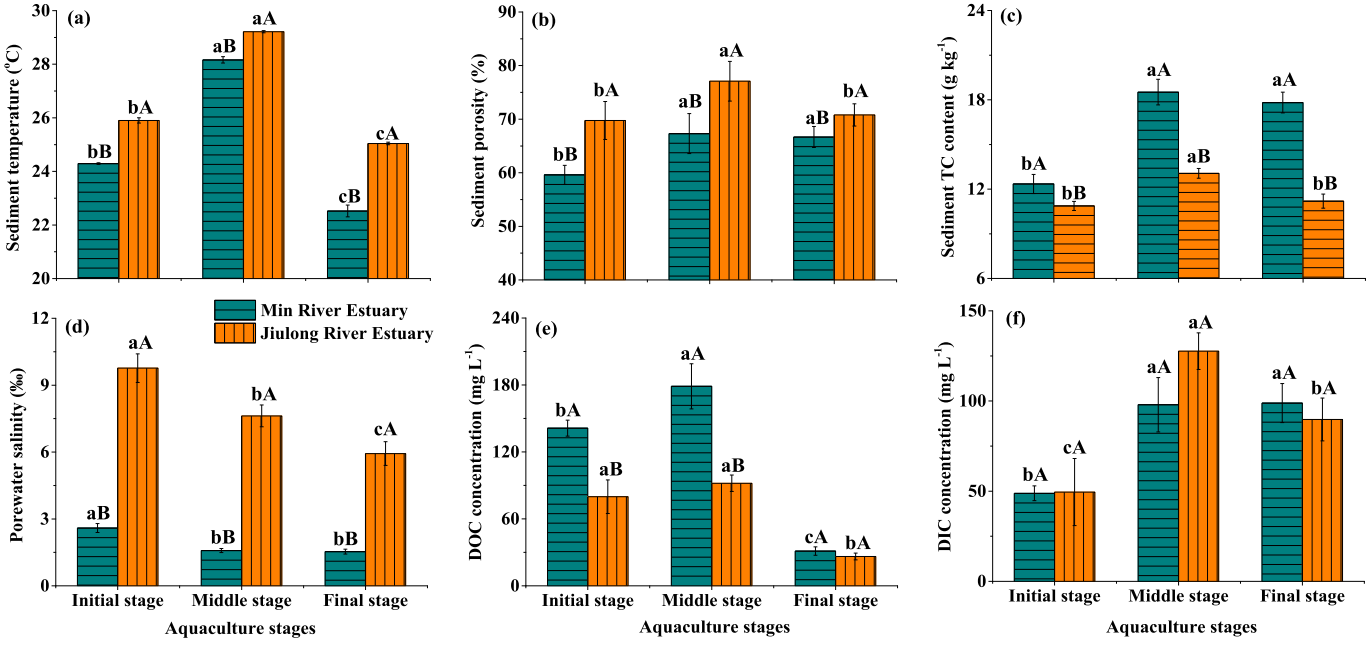

Fig. 2. Variations in (**a**) temperature, (**b**) porosity, and (**c**) total carbon (TC) in surface (0–15 cm) sediments, and (**d**) salinity, (**e**) dissolved organic carbon (DOC), and (**f**) dissolved inorganic carbon (DIC) concentrations in the sediment porewater of shrimp ponds at the Min River Estuary (MRE) and Jiulong River Estuary (JRE). Bars represent means ± 1 SE (n = 9). Different lowercase and uppercase letters on the bars indicate significant differences among different growth stages and estuaries, respectively ($p < 0.05$).

used to screen the major influential environmental factors for the temporal variations of CO_2 production rates in overlying water and CO_2 fluxes across the SWI from different estuarine ponds. All statistical analyses were performed using the SPSS statistical software package (SPSS v 22.0, IBM, Armonk, NY, USA) and the statistical results at the level of 0.05 were considered as significance. The results were presented as average ± 1 standard error. Statistical plots and conceptual diagrams were generated using OriginPro 7.5 (OriginLab Corp. USA).

3. Results and discussion

3.1. Surface sediment, porewater, and overlying water characteristics

The characteristics of surface sediments in the shrimp ponds of the two estuaries over the study period are shown in Fig. 2a–c. The minimum and maximum sediment temperatures were recorded at initial and middle stages, respectively. Significant differences in mean temperature were detected between the two estuaries at all three periods ($p < 0.05$) (Fig. 2a). Sediment porosity at MRE was significantly lower at the initial stage than middle and final stages ($p < 0.05$) (Fig. 2b), while at JRE, significantly higher porosity was observed during the middle stage ($p < 0.05$) (Fig. 2b). The average sediment porosity was significantly higher at JRE than MRE over the study period ($p < 0.01$). A similar seasonal trend was observed for sediment TC content in the shrimp ponds, with considerably smaller and larger results at initial and middle stages, respectively (Fig. 2c). Although the temporal patterns of sediment TC at MRE and JRE were very similar, significant differences in mean values were observed between the two estuaries in all three periods ($p < 0.01$) (Fig. 2c). Meanwhile, the mean TC values found in our ponds were within the range of 1.08–5.43% observed across 233 aquaculture ponds around the world (Boyd et al., 2010). The mean sediment pH values at ponds in MRE and JRE were 6.9 ± 0.1 and 6.3 ± 0.1, respectively. The seasonal changes of sediment pH at ponds in two estuaries followed the order of initial stage < final stage < middle stage.

The characteristics of sediment porewater in the shrimp ponds are shown in Fig. 2d–f. The temporal patterns of porewater salinity were very similar in the two estuaries, with a generally decreasing trend over the study period (Fig. 2d). Due to a greater input of freshwater (e.g., terrestrial/estuarine groundwater, precipitation), JRE had significantly higher porewater salinity than MRE ($p < 0.01$). Porewater DOC concentration reached a minima and a maxima in October and August, respectively, while significant differences in mean DOC concentrations between the two estuaries were detected in both June and August ($p < 0.01$) (Fig. 2e). The lowest and highest porewater DIC concentrations were detected in June and August, respectively, with no significant differences between the two estuaries at all times (Fig. 2e). The variations in various environment variables in the overlying water during the shrimp growth cycle are shown in Fig. 3. The bottom water had pH values ranging 8.4–10.2 at MRE and 8.2–9.8 at JRE (Fig. 3a), with significant differences between estuaries at initial and final stages ($p < 0.05$), and significantly lower pH at middle stage ($p < 0.05$). DO concentrations in the pond water of both estuaries showed an increasing trend over time (Fig. 3b). The concentrations of DOC and DIC in JRE ponds increased with time, while those in MRE ponds were significantly higher during the middle stage ($p < 0.05$; Fig. 3c and d). The mean water DOC and DIC concentrations at MRE ponds were 12.6 ± 0.3 mg L^{-1} and 21.9 ± 1.2 mg L^{-1}, respectively, which were significantly higher than those at JRE ponds (6.7 ± 0.4 mg L^{-1} and 16.2 ± 0.7 mg L^{-1}, respectively, $p < 0.01$).

3.2. Porewater dissolved CO_2 concentrations

The concentrations of dissolved CO_2 in the sediment porewater are

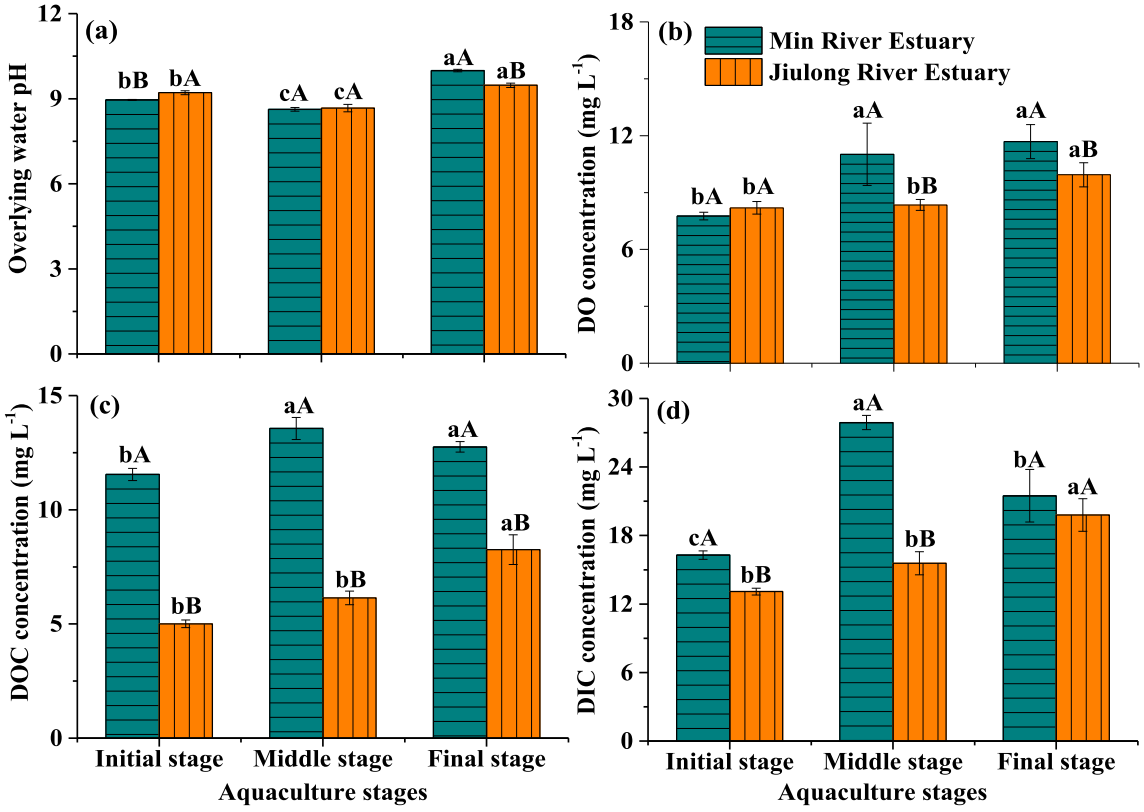

Fig. 3. Variations in (**a**) pH, (**b**) dissolved oxygen (DO), (**c**) dissolved organic carbon (DOC) and (**d**) dissolved inorganic carbon (DIC) in the overlying water of shrimp ponds at the Min River Estuary and Jiulong River Estuary. Bars represent means ± 1 SE ($n = 9$). Different lowercase and uppercase letters on the bars indicate significant differences among different growth stages and estuaries, respectively ($p < 0.05$).

shown in Fig. 4a. The mean porewater CO_2 concentrations at MRE and JRE ponds during the study period were 4.9 ± 0.6 and 2.1 ± 0.6 mg L^{-1}, respectively, with a range of 4.4–6.0 and 0.9–3.1 mg L^{-1}, respectively. The porewater CO_2 concentrations at MRE and JRE demonstrated similar seasonal trends, with higher and lower values at the middle and final stages, respectively (Fig. 4a and Table 1). CO_2 concentration shared the similar patterns with temperature and porewater DOC in sediments ($p < 0.05$ or $p < 0.01$) (Fig. 2a and e, and Table 2), indicating that temperature, organic matter, and their interactions were important factors influencing the variability of porewater CO_2 concentrations in shrimp ponds. The CO_2 in porewater was predominantly produced by the degradation of organic matter (Gruca-Rokosz and Tomaszek, 2015; Wollast, 1993). The presence of a large amount of organic matter would supply a large amount of substrates to microbes for the soil C mineralization (Kristensen et al., 2008), with the possible consequence of increase in porewater CO_2 levels. Furthermore, a higher temperature could greatly enhance

Table 1
Results of two-way ANOVA of the effects of estuaries and aquaculture stages on sediment porewater CO_2 concentrations, CO_2 production rates, and CO_2 fluxes across the SWI of shrimp ponds at the Min River Estuary and Jiulong River Estuary.

	df	Porewater CO_2	CO_2 production	CO_2 fluxes
Estuaries	1	67.240**	18.674**	1.980
Aquaculture stages	2	10.162*	17.889**	33.557**
Aquaculture stages × Estuaries	2	1.700	4.513*	3.094

**$p < 0.001$, *$p < 0.01$.

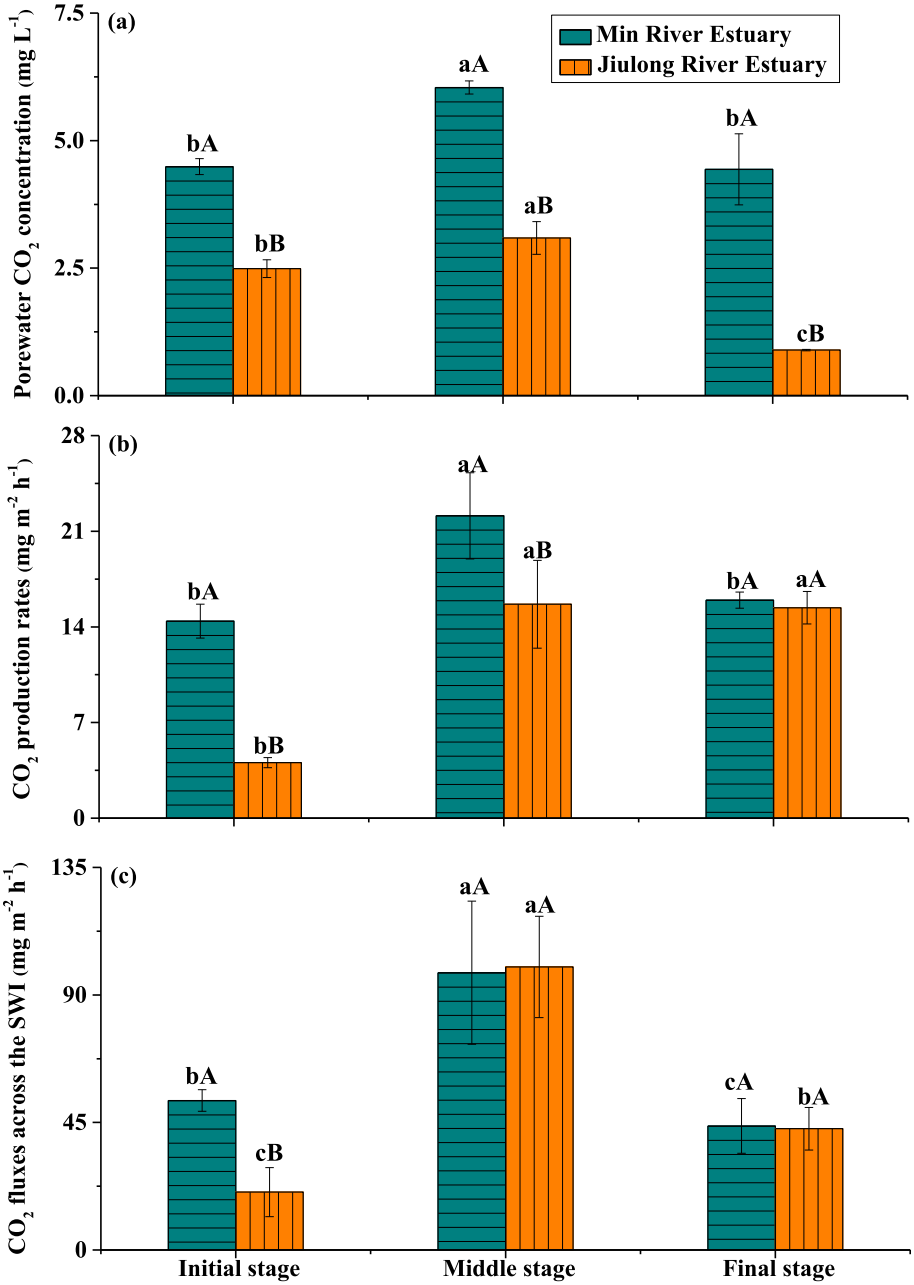

Fig. 4. Variations in (**a**) sediment porewater CO_2 concentration, (**b**) overlying water CO_2 production rate (CO_2_WP), and (**c**) CO_2 fluxes across the sediment-water interface (SWI) of shrimp ponds at the Min River Estuary and Jiulong River Estuary. Bars represent means ± 1 SE ($n = 9$). Different lowercase and uppercase letters on the bars indicate significant differences among different growth stages and estuaries, respectively ($p < 0.05$).

Table 2
Pearson correlation coefficients for porewater CO_2 concentrations and various physico-chemical parameters of porewater and sediments in the shrimp ponds of the Min River Estuary (MRE) and Jiulong River Estuary (JRE).

Estuary	Porewater			Sediment			
	DOC	DIC	Salinity	Temperature	Porosity	TC	pH value
MRE	0.451*	NS	NS	0.398*	NS	NS	−0.392*
JRE	0.486**	NS	NS	0.672**	NS	0.411*	−0.528**

NS denotes "nonsignificant relationship". [a] $n = 27$ for environmental variables and porewater CO_2 concentrations at shrimp ponds in each estuary. The symbols * and ** denote significant correlations at $p < 0.05$ and 0.01, respectively.

microbial decomposition of soil organic matter (Golovatskaya and Dyukarev, 2009; Lafleur et al., 2005), and thus the release of CO_2 from sediments into the porewater. Our results suggested that the high sediment temperature and the large organic matter associated with the high bait feeding and intense metabolic activity of shrimps (Burford et al., 2003) would contribute to the elevated porewater CO_2 concentrations observed in the middle stage compared to initial and final stages. The strong and negative correlations found between pH and porewater CO_2 concentrations (Table 2) further suggested that the temporal variations in sediment porewater CO_2 concentrations might partly depend on pH changes. Our results were in agreement with those of previous studies conducted in aquatic ecosystems (e.g. Crawford et al., 2013; Neal et al., 1998; Wallin et al., 2010), especially in shallow aquaculture ecosystems (Chen et al., 2016; Xiong et al., 2017).

Porewater CO_2 concentrations varied significantly between MRE and JRE across these three shrimp growth stages ($p < 0.001$) (Table 1), with generally high and very low values for MRE and JRE ponds, respectively (Fig. 4a). One possible reason for this spatial variations could be attributed to the differences in sediment TC contents and porewater DOC concentrations (Fig. 2c and e, and Table 1), which was in line with the earlier discussion of the effects of organic matter on sediment CO_2 production. In the present study, MRE ponds had much lower porewater salinity than JRE ponds (Fig. 2d), which could be a result of freshwater dilution caused by the interactions between precipitation and surface runoff. Combining two estuaries together, porewater CO_2 concentration were negatively correlated with porewater salinity ($r = 0.54$, $p < 0.01$), indicating that salinity was another important factor influencing the porewater CO_2 concentrations in the estuarine ponds. High salinity has been suggested to inhibit the activities of, or even bring harm to, microorganisms, which would subsequently reduce

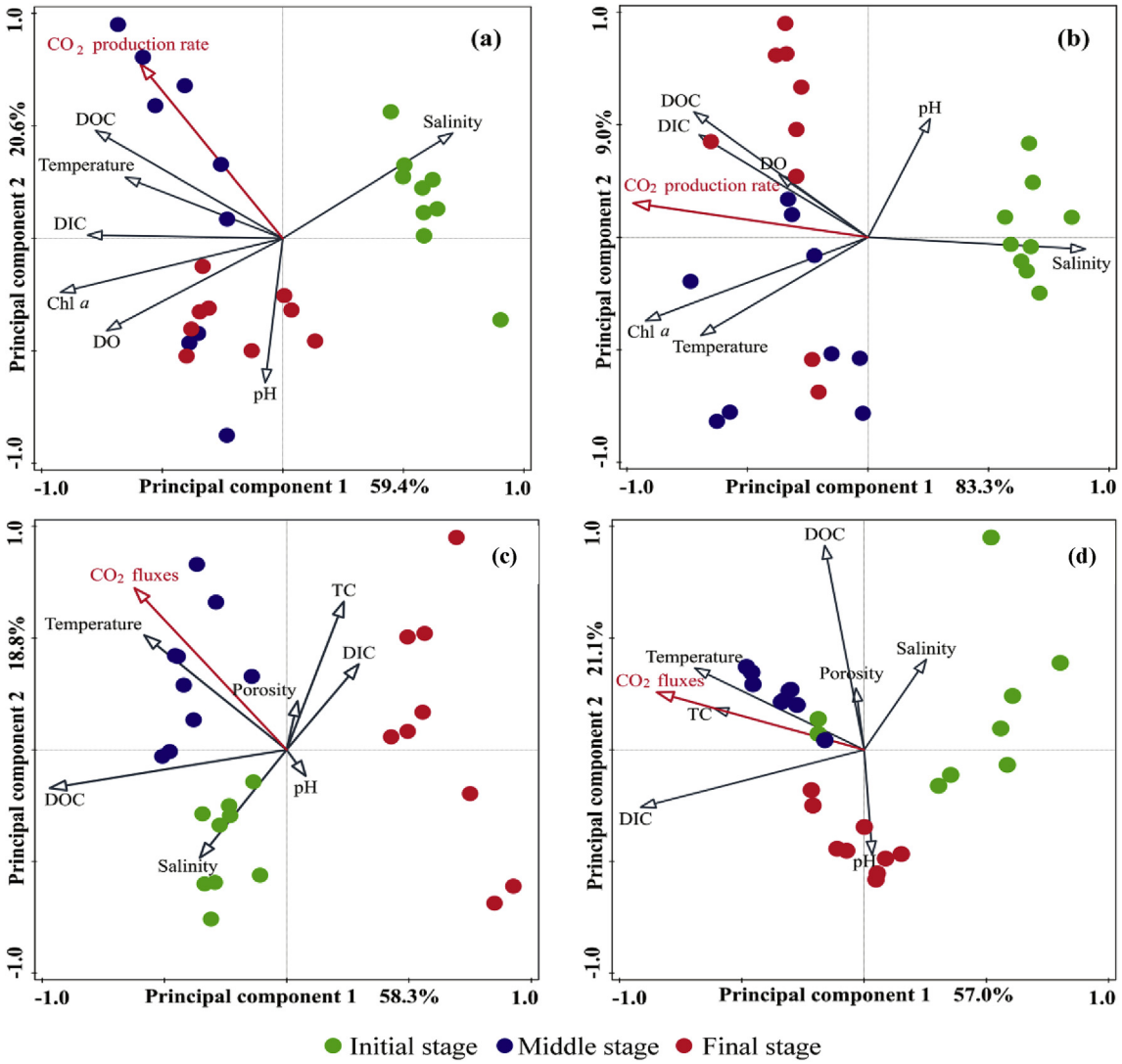

Fig. 5. The principal component analysis biplots of the CO_2 production rates in the overlying water, CO_2 fluxes across the SWI and various environmental factors of (**a, c**) Min River Estuary and (**b, d**) Jiulong River Estuary ponds, showing the loadings of environmental factors (arrows) and the scores of observations in different aquaculture stages (points).

carbon mineralization rates and CO_2 production (Hu et al., 2017).

3.3. Production rates of CO_2 in overlying water

The culture of aquatic fauna in mariculture ponds is supported by daily supply of feeds (Chen et al., 2016). These ponds accumulate a large amount of organic carbon from residual feeds and feces (Burford et al., 2003; Chen et al., 2016), which supported significant CO_2 production arising from the microbial decomposition of organic matter in the aerobic water column and subsequently the release of CO_2 from aquaculture ponds into the atmosphere as shown by our data. Fig. 4b shows our laboratory incubation experiment results about the production rates of CO_2 in the overlying water. There was a clear temporal variation of CO_2 production rates in all the mariculture ponds during the study period ($p < 0.001$, Table 1). The CO_2 production rates in MRE ponds ranged between 14.5 and 22.0 mg m^{-2} h^{-1}, with significantly smaller values during the initial and final stages than middle stage ($p < 0.01$). The CO_2 production rates in JRE ponds ranged 3.9–15.8 mg m^{-2} h^{-1}, with significantly lower results at the initial stage ($p < 0.01$, Fig. 4b). Chen et al. (2015) observed similar temporal patterns in grass carp *Ctenopharyngodon idella* polyculture ponds, and suggested that temperature, Chl-*a* (Chlorophyll *a*) concentrations, and water temperature played a primary role in controlling CO_2 production. Some studies also reported a considerable temporal variability in CO_2 production rates in the freshwater environment and wetland sediments (e.g. Almeida et al., 2016; Vachon et al., 2016; Weyhenmeyer et al., 2015), which was mainly governed by seasonal variability in temperature and organic matter concentrations (Hu et al., 2017; Vachon et al., 2016). However, the results of our principal component analysis showed that the temporal variations in CO_2 production rates were primarily related to different sets of environmental variable between sites (Fig. 5). In MRE ponds, the CO_2 production rate was significantly related to DOC ($p < 0.01$) and temperature ($p < 0.01$) (Fig. 5a), which together accounted for 76.1% of the variance in CO_2 production rates (Table 3). In JRE ponds, however, the temporal patterns of overlying water CO_2 production rates were mainly driven by salinity ($p < 0.01$) and DIC concentration ($p < 0.01$) (Fig. 5b), which together accounted for 68.7% of the variance in CO_2 production (Table 3). Salinity was a significant factor affecting CO_2 production in JRE but not MRE, which could be related to the much lower baseline salinity level and hence a greater sensitivity of microbial activities to salinity changes in the former estuary. In contrast, CO_2 production in the more saline MRE ponds was more strongly controlled by the supply of organic substrates (e.g. DOC) for microbial mineralization.

The production rates of CO_2 in the overlying water also varied markedly between the two estuaries (Fig. 4b). The mean CO_2 production rate in MRE was significantly higher than that in JRE ponds (17.6 ± 1.3 vs. 10.6 ± 1.3 mg m^{-2} h^{-1}, $p < 0.001$, Table 1). The conversion of natural coastal wetlands to aquaculture ponds conceals or eliminates the spatial heterogeneity, topographic features, and hydrological characteristics of the habitats (Yang et al., 2017b). However, the magnitude of chemical parameters measured in the pond overlying water varied significantly between the two estuaries. In particular, significant differences in salinity, DOC, and DIC concentrations between MRE and JRE ponds were observed ($p < 0.05$ or $p < 0.01$) (Figs. 2d, 3c and d). The survival rate and the densities of shrimps and baits were the major factors affecting DOC and DIC concentrations in mariculture ponds (Yang et al., 2018a). Considering high density of shrimps, a large amount of bait was added into the ponds in MRE. However, the low survival rate of shrimps (MRE vs. JRE: 65% vs 71%) had resulted in a substantial accumulation of surplus baits, which would decompose and contribute to high DOC and DIC concentrations in the water column (Fig. 3c and d). In addition, the lower salinity in MRE arising from the greater amount of freshwater runoff could contribute to the enhanced CO_2 production in the ponds (Fig. 4b). In contrast, the JRE ponds had a higher water salinity but a lower bait concentration as a result of a lower density and a higher survival rate of shrimps, which together led to both lower organic matter content and CO_2 production rates as compared to MRE (Fig. 4b). Our results showed that local environmental conditions affecting the physico-chemical properties of shrimp pond water (e.g. organic matter, salinity, and others) were important driver causing the observed difference in CO_2 production rates between ponds.

3.4. Fluxes of CO_2 across the sediment-water interface

CO_2 fluxes across the SWI are shown in Fig. 4c. The relationships between CO_2 fluxes and the environmental variables are also shown in two separate PCA plots, which demonstrated the temporal patterns in MRE (Fig. 5c) and JRE ponds (Fig. 5d), respectively. The CO_2 fluxes from ponds in MRE and JRE were high, ranging between 43.6 and 97.7 mg m^{-2} h^{-1} and between 20.2 and 99.9 mg m^{-2} h^{-1}, respectively. Significant differences in mean CO_2 fluxes were recorded among the three shrimp growth stages ($p < 0.001$, Table 1) and the highest values was detected in the middle stage (Fig. 4c). The temporal variations in pond CO_2 fluxes between the two estuaries were influenced similarly by sediment temperature (MRE: $R^2 = 0.38$, $p < 0.01$; JRE: $R^2 = 0.69$, $p < 0.01$; also see Fig. 5 and Table 4) and sediment TC level (MRE: $R^2 = 0.17$, $p < 0.05$; JRE: $R^2 = 0.31$, $p < 0.01$), implying that the importance of temperature for the mineralization of organic matter and CO_2 fluxes across the SWI (Table 4 and Fig. 5). Xiong et al. (2017) also reported that an increase in temperature could stimulate soil microbial activities and carbon mineralization, resulting in an oversaturation of CO_2 and hence a large release of CO_2 from the sediments to the overlying water. Moreover, this study found that the temporal changes in CO_2 fluxes across the SWI were similar to those of the gradient of CO_2 concentrations between the porewater and overlying water over the study period (Fig. 6), indicating that the CO_2 diffusive gradient could at least in part govern the variations in CO_2 flux among the three stages of shrimp growth. Some recent studies further suggested that *L. vannamei* in the aquaculture ponds could affect carbon transport and transformation in surface sediments because the depth of sediment bioturbation caused by this shrimp was up to 2 cm (Xiong et al., 2017; Zhong et al., 2015). The different intensities of bioturbation among the three shrimp growth stages might also contribute to the observed seasonal changes of CO_2 fluxes across the SWI.

Over the study period, the mean CO_2 flux across the SWI in the MRE ponds was 63.68 ± 6.56 mg m^{-2} h^{-1}, greater than that in the JRE ponds (54.36 ± 7.70 mg m^{-2} h^{-1}). The variation patterns of CO_2 flux and porewater CO_2 concentration were highly similar (Fig. 4a and c; $r^2 = 0.40$, $p < 0.001$), which is consistent with that of previous findings that high GHG emissions are associated with high porewater GHG concentrations (Tong et al., 2018; Xiang et al., 2015; Zhang et al., 2010b). The magnitude of CO_2 fluxes from our estuarine ponds was also different from that of other aquatic ecosystems (Table 5). The average values and the range of CO_2 fluxes observed in our shrimp pond systems were substantially larger than those reported in lakes (Casper et al., 2003; Liikanen et al., 2002; Ogrinc et al., 2002; Yang et al., 2015a) and reservoirs (Gruca-Rokosz et al., 2011; Gruca-Rokosz and Tomaszek, 2015). The CO_2 fluxes across the SWI from the shrimp ponds in our two estuaries were also higher than those from the subtropical rivers or

Table 3
Results of stepwise multiple linear regression analysis between CO_2 production rates and various environmental parameters in the overlying water of the Min River Estuary (MRE) and Jiulong River Estuary (JRE).

Estuary	Regression equations	*F*-value	R^2	*p*-value
MRE	$Y = 3.949X_{DOC} - 2.636X_{pH} - 8.105$	38.161	0.761	< 0.001
JRE	$Y = -1044X_{salinity} + 0.672X_{DIC} + 10.373$	26.360	0.687	< 0.001

Table 4
Results of stepwise multiple linear regression analysis between CO_2 fluxes across the SWI and various environmental parameters in the pond sediments of the Min River Estuary (MRE) and Jiulong River Estuary (JRE).

Estuary	Regression equations	*F*-value	R^2	*p*-value
MRE	$Y = 8.184X_{Temperature} - 0.502X_{DIC} + 3.555X_{TC} - 152.525$	15.006	0.662	< 0.001
JRE	$Y = 22.129X_{Temperature}$ - 546.524	56.542	0.693	< 0.001

estuaries, such as the Mississippi River Estuary, USA (Morse and Rowe, 1999) and the Shanghai river network, China (Tan, 2014), but generally lower than those from the drainage ditches in the Netherlands (Schrier-Uijl et al., 2011) and the intertidal mudflats in Japan (Kikuchi, 1986). Meanwhile, the magnitude of CO_2 fluxes in our study was similar to that in the freshwater aquaculture systems in China (Xiong et al., 2017), which suggested that the sediments of mariculture ponds could be potential "hotspots" of CO_2 emissions, and the role of mariculture ponds should be taken into account when evaluating the CO_2 balance of aquatic ecosystems.

The high CO_2 fluxes across the SWI observed in our shrimp ponds were, to some extent, related to the large supply of organic matter. These mariculture ponds were often maintained through daily feed application to culture the target aquatic fauna (Chen et al., 2015, 2016). In fact, the feed utilization efficiency is unfortunately as low as 4.0–27.4% (Chen et al., 2015; Molnar et al., 2013), so only a limited proportion of the feed inputs could be converted into shrimp biomass. Very likely majority of the added feeds would end up accumulating in the mariculture systems (Chen et al., 2015; Yang et al., 2017a), leading to the generation of a large amount of organic residues, mainly uneaten feeds, during mariculture production that in turn stimulate microbial decomposition and subsequently CO_2 production and emission from mariculture ponds.

3.5. Implications and future outlook for carbon biogeochemical cycling

3.5.1. Role of sediment in the internal CO_2 production of shrimp ponds

Decomposition or mineralization of organic matter plays a crucial role in the internal CO_2 production in aquatic ecosystems (Müller et al., 2016; Vreča, 2003). According to Weyhenmeyer et al. (2015), the internal CO_2 production (CO_{2_IP}) comprised of three different processes, namely CO_2 production at the sediment by microbial mineralization (CO_{2_SP}), CO_2 production in the water column by microbial mineralization of DOC (CO_{2_WP}), and CO_2 production in the water column by photochemical mineralization (CO_{2_PP}). Numerous studies reported that CO_{2_WP} often made the largest contribution to the internal CO_2 production in the boreal lakes (e.g. Almeida et al., 2016; Brothers et al., 2012; Weyhenmeyer et al., 2015). Weyhenmeyer et al. (2015) reported that the median CO_2 production rate in over 5000 boreal lakes generally followed the order of CO_{2_WP} (221 mg C m^{-2} d^{-1}) > CO_{2_SP} (47 mg C m^{-2} d^{-1}) > CO_{2_PP} (25.6 mg C m^{-2} d^{-1}), with significantly higher values in autumn. Similarly, Algesten et al. (2005) reported that CO_{2_SP} played a minor role in the production and emissions of CO_2 in the boreal and subarctic lakes in the summer. In the present study, CO_2 release fluxes across the SWI and CO_{2_WP} in the shrimp ponds ranged between 20.2 and 99.9 mg m^{-2} h^{-1}, and between 3.9 and 22.0 mg m^{-2} h^{-1}, respectively. The mean CO_2 release fluxes across the SWI in the two estuarine ponds was over 3.9 times larger than the CO_{2_WP} rate (58.9 vs. 14.9 mg m^{-2} h^{-1}, $p < 0.01$), which suggested that sediment CO_2 production contributed substantially to the total production and emissions of CO_2 in subtropical aquaculture ponds during the culture period. The relative larger contribution of CO_{2_SP} to the CO_{2_IP} in subtropical shrimp ponds is different from that in the boreal and subarctic lakes. There were two possible mechanisms that could account for the considerable impacts of sediments on the CO_2 balance in the current study. First, the pond sediment received a large quantity of organic carbon from feed residuals and feces (Chen et al., 2016; Yang et al., 2017a) that served as important substrates to microbes for decomposition and subsequent CO_2 production. Second, the shallow water depth (an average of 1.5 m) and the operation of paddlewheel aerators would effectively promote the diffusion of oxygen from the water into the sediment (Silva et al., 2013; Yang et al., 2017a), which in turn enhance aerobic respiration and the microbial decomposition of sediment organic matter.

3.5.2. Impact of pond effluent on CO_2 dynamics in receiving coastal waters

In the end of aquaculture production, pond water is complete drained to discard the aquaculture wastewater and aerate the bottom sediments to prepare for the next production (Herbeck et al., 2013; Yang et al., 2017a). During this management practice, large quantities of nutrient-enriched water would be transferred into the adjacent coastal zone over a short period (Wu et al., 2014), with a serious consequence of water pollution, eutrophication and other damages to the environment (Yang, 2014). Annual discharge of total nitrogen (TN) and total phosphorus (TP) from shrimp mariculture into the Min River Estuary was estimated to 30.45 and 2.40 Mg, respectively (Yang et al., 2017a). Consequently, TN and TP concentrations in the receiving waters jumped markedly by 270% and 234%, respectively (Yang et al., 2017a). This study further estimated that the annual discharge of DOC and DIC from shrimp mariculture into the adjacent coastal waters was 444.1 and 706.2 Mg, respectively, for MRE (the total pond area of 26.30 km^2, water depth of 1.4 m, and mean DOC and DIC concentrations of 13.5 and 21.5 mg L^{-1}, respectively) and 550.2 and 1264.1 Mg, respectively, for JRE (a total pond area of 43.3 km^2, water depth of

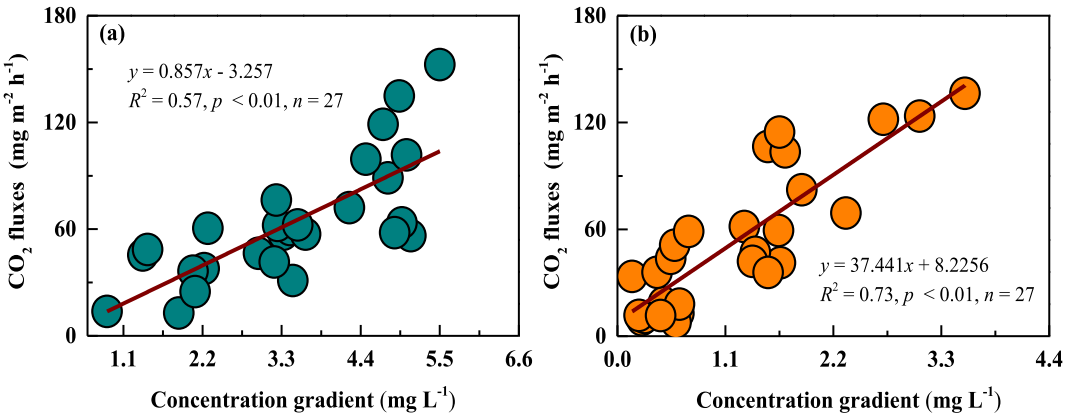

Fig. 6. Relationships between CO_2 fluxes and CO_2 concentration gradients across the SWI of shrimp ponds at the (**a**) Min River Estuary and (**b**) Jiulong River Estuary. The solid lines represent the best-fit linear regression ($p < 0.01$).

Table 5
A summary of CO_2 fluxes (mg m^{-2} h^{-1}) across the sediment-water interface in different aquatic ecosystems (e.g. lakes, reservoirs, rivers, drainage ditch, aquaculture ponds, and others).

Ecosystems Type	Study Site	Average Depth (m)	Range	Reference
Lake	Bled Lake, Slovenia	17.9	9.2	Ogrinc et al. (2002)
	Stechlin Lake, Germany	22.8	4.4–6.2	Casper et al. (2003)
	Kevatön Lake, Finland	2.3	20.2–56.8	Liikanen et al. (2002)
	Baldeg Lake, Switzerland	56–65	3.9–13.2	Urban et al. (1997)
Reservoir	Wilcza Wola Reservoir, Poland	2.6	2.2–3.9	Gruca-Rokosz et al. (2011)
	Solina Reservoir, Poland	22.0	2.2–2.6	Gruca-Rokosz et al. (2011)
	Rzeszów Reservoir, Poland	0.5–6.0	0.9–83.2	Gruca-Rokosz and Tomaszek (2015)
	Lobo Broa Reservoir, Brazil	3.0	16.3–47.9	Adams (2005)
River/Estuary	Mississippi River estuary	–	31.2–102.5	Morse and Rowe (1999)
	Kertinge Nor River estuary	–	128.5–155.8	Hansen and Kristensen (1997)
	Shanghai river network, China	1.35–4.0	−43.6–52.8	Tan, 2014
Intertidal mudflat	Nanakita River, Japan	–	117.0–533.7	Kikuchi (1986)
Drainage ditch	Netherlands	0.25–0.90	69.5–198.9	Schrier-Uijl et al. (2011)
Aquaculture pond	Shandong Province, China	1.8	19.8–124.1	Xiong et al. (2017)
	Min River estuary, China	1.3	43.6–97.7	Present study
	Jiulong River estuary, China	1.5	20.2–99.9	Present study

"–" indicated No data.

1.5 m, and mean DOC and DIC concentrations of 8.3 and 18.9 mg L^{-1}, respectively). Considering the total area of China's subtropical estuaries mariculture of 6.6 × 10^3 km^2 (Yao et al., 2016) and a mean water depth of 1.4 m and assuming that our data were representative of the mariculture ponds across China, it is estimated that approximately 100 Gg DOC y^{-1} and 190 Gg DIC y^{-1} would be directly discharged from the mariculture ponds into the adjacent coastal zone. The decomposition of organic matter such as DOC was the main driver of the internal CO_2 production in aquatic ecosystems (Müller et al., 2016; Weyhenmeyer et al., 2015). The discharge of aquaculture effluents can rapidly alter the supply of organic matter and the quality of nearby waters (Herbeck et al., 2013; Yang et al., 2017c), which subsequently create a favorable environment for CO_2 production internally in the coastal ecosystems. Our results pointed to the potential of adjacent receiving coastal waters in the effluent discharge area of the ponds being potential "hotspots" of CO_2 emissions in winter.

3.5.3. Management to reduce CO_2 emission from aquaculture ponds

Yang et al. (2018a,b) found variations in CO_2 emissions fluxes across the water-air interface from aquaculture ponds among estuaries, with high fluxes in Min River Estuary (17.47 mg m^{-2} h^{-1}) and low fluxes in Jiulong River Estuary (15.40 mg m^{-2} h^{-1}). The variation of CO_2 emission fluxes was similar to those of porewater CO_2 concentrations (Fig. 4a), overlying water CO_2 production rates (Fig. 4b) and sediment CO_2 release rates (Fig. 4c). The results indicate that high CO_2 emissions were accompanied by high CO_2 production and porewater CO_2 concentrations. The high variation of CO_2 emission and other biogeochemical processes from mariculture ponds is commonly related to multiple environmental factors, but low salinity is necessary to produce high CO_2 production and emission fluxes. This implies that increasing salinity level of aquaculture ponds might be a measure to reduce CO_2 emission from aquaculture, but the potential impact on other environmental conditions should be monitored and minimized. From 1 January 2015, China has started the new Environmental Protection Law (EPL) and the strict implementation of EPL and related regulations will be the key for the sustainable development of aquaculture in China (Yang, 2014; Yang et al., 2015b).

3.5.4. Limitation and future research

Similar as many studies, there are some limitations in the current study. It should be noted that a certain level of uncertainties might exist in our estimated contributions of CO_{2_SP} and CO_{2_WP} to the overall CO_{2_IP} rate of aquaculture ponds owing to the limited size of our data sets with only two estuaries. Future research should increase the frequency of *in situ* sampling, for example the diurnal change (Xing et al., 2004), and include more innovative techniques to measure CO_{2_SP} and CO_{2_WP} in aquaculture ponds at multiple spatial scales. Future long-term *in situ* sampling and monitoring with multiple frequencies in various regions of China should be carried out to obtain a more complete picture of the influence of aquaculture pond effluents on CO_2 production and emissions in coastal ecosystems. Furthermore, the aquaculture pond ecosystem is characterized by shallow water depth, high transparency, and high daily feed supply, which together could provide a suitable environment for CO_2 production in the water column by photochemical mineralization (CO_{2_PP}) and heterotrophic respiration of shrimps (CO_{2_SR}). While CO_{2_PP} and CO_{2_SR} are likely important contributors to the internal CO_2 production in subtropical aquaculture ponds, the roles of CO_{2_PP} and CO_{2_SR} deserve further investigation. In the current study, CO_2 fluxes were analyzed completely based on laboratory incubation experiments, and further *in situ* experiments will be helpful to unravel the detailed mechanisms of bioturbation on CO_2 efflux.

4. Conclusions

Carbon dioxide production from sediment and overlying water at aquaculture shrimp ponds in two subtropical estuaries were research in the current study. Significant differences in porewater CO_2 concentrations, CO_{2_WP}, and CO_2 fluxes across the SWI were observed at the shrimp ponds in subtropical estuaries among growth stages, with much higher values in the middle stage. Our results suggested that the seasonal variations in sediment temperature and organic matter supply were the key drivers of the changes in porewater CO_2 concentrations and CO_2 fluxes, while the temporal variations of CO_{2_WP} were governed by the interactions between organic matter and other abiotic factors (e.g. pH and salinity). Higher porewater CO_2 concentrations and CO_{2_WP} in the MRE than the JRE ponds could be partly attributed to the difference in salinity levels between the two estuaries. Our results further highlighted the importance of considering the variability of CO_2 production among different estuaries and aquaculture stages in order to produce reliable extrapolation and estimates of large-scale carbon balances. The mean CO_2 fluxes across the SWI in the ponds was approximately 3.9 times larger than the mean CO_{2_WP} rate, suggesting that sediment was an important contributor to the internal CO_2 production at the shrimp ponds in subtropical estuaries. Therefore, formulating management strategies in minimizing sediment CO_2 release would be crucial for reducing CO_2 emissions from aquaculture ponds to the atmosphere in future.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2019.01.088.

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